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Persistence of capsicum oleoresin in soil

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Abstract

We studied the persistence of capsicum oleoresin in a sandy loam soil. Five capsicum–soil concentrations (6.00%, 3.00%, 1.50%, 0.75%, and 0.00% wt : vol) were prepared in separate plastic boxes without lids; soils were elevated above wire-screen sub-floors to allow percolation and were exposed outdoors to ambient conditions. Plexiglass shields were placed above the boxes to allow ventilation but to deflect precipitation. During an initial 14-day period, soils were sprinkled once daily with 0.64 ml/cm^2 of water to simulate rainfall, then were maintained dry for a final 14 days. Spectrophotometric measurements (282 nm capsaicin wavelength) of independent, dual, oven-dried, 10-g samples from each soil mixture were made for Days 0 (pre-watering), 2, 4, 6, 8, 10, 12, 14, and 28 post mixing. Results showed that: (1) simulated rainfall was an important linear regressor of capsicum oleoresin in a sandy loam soil accounting for 43–75% of spectrophotometric variation, (2) mean λ_{max} absorbance (282 nm) of soil extracts decreased $\geqslant$45% after cumulative sprinkling with 8.96 cm of water (14 days of 0.64 cm/day), then remained essentially unchanged during a subsequent 14 days without simulated rainfall, and (3) regression slopes for the initial 14-day mean λ_{max} absorbance functions for the 4 oleoresin–soil mixtures were heterogeneous (i.e., differed in slope), with greater concentrations showing relatively faster depletion. Thus, capsicum oleoresin persisted in soil for $\geqslant$28 days, with persistence enhanced under arid conditions. Although costs of capsicum oleoresin are prohibitive, these persistence data support the feasibility of developing "in-soil irritant" technology to repel fossorial rodents from prescribed areas—a less expensive, persistent irritant is sought. Published by Elsevier Science Ltd.

1. Introduction

Capsaicin-based products have gained diverse uses as animal repellents (see Mason, 1997; Baker et al., 1999); examples of registered compounds include: Counter Assault®, Deer Away®, Hinder® and Hot Sauce®. Repellency in animals requires that the irritant is delivered in sufficient concentration/exposure so as to permeate tissue (e.g., oral, nasal, mucosa, dermal) and to depolarize nociceptive nerve endings (Bryant, 1999).

The US Environmental Protection Agency (EPA) lists capsaicin (CAS No. 404-86-4) as a biochemical pesticide —a naturally occurring, non-carcinogenic substance with a non-toxic (irritant) mode of action (US Environmental Protection Agency, 1992a, b). The active ingredient (A.I.) is obtained by grinding dried, ripe chili peppers (*Capsicum frutescens L.*); whereas, the oleoresin (CAS No. 8023-77-6) is made by distilling the powdered A.I. in a solvent and evaporating the solvent. Little product chemistry or environmental fate (e.g., sorption) data exist for either capsaicin or capsicum oleoresin—a probable effect of the assumed non-toxic properties of the plant extracts and the lack of data requirements for these product registrations (US Environmental Protection Agency, 1992a). A search of 36 databases yielded few articles dealing with physical chemistry or environmental fate data related to capsaicin or capsicum oleoresin (A. Noble and J. Eisemann, Pers. Comm., 2001). Articles of relevance concerned the observation that the bacteria, *Pseudomonas maltophilia*, speeded decomposition of capsaicin to vanilyllamine (see Onozaki et al., 1985, 1986).

Recently, Sterner et al. (1999) reported that capsicum oleoresin soil mixtures $\geqslant$1.50% (wt : vol) reduced soil-contact time by northern pocket gophers (*Thomomys talpoides*) ~50% relative to animals exposed to control soil (0% capsicum oleoresin). Capsicum–soil-exposed gophers also spent more time grooming their pelage which indirectly reduced digging times and numbers of digging bouts. The results imply that gopher avoidance of prescribed areas may be induced by dispensing capsicum oleoresin in tunnel systems and adjacent soils. A main issue affecting development of this "soil-irritant" approach to gopher repellency

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concerns the persistence of capsicum oleoresin in soil—maintenance of sufficient irritant in a soil medium to cause pain. Here, we report results of a 28-day parametric study to determine the persistence of capsicum oleoresin in a sandy loam soil.

2. Materials and methods

2.1. Soil

Topsoil ($\leqslant$30 cm depth) was scooped from a field near Wellington, Colorado. Analysis showed that this was a sandy loam soil (62% sand, 26% silt, and 12% clay), with 7.9 pH and 2.5% organic matter (Agvise Laboratories, Northwood, ND). All soil was dried 1–2 days prior to use by placing it onto metal trays (< 2.5-cm depth) in a laboratory drying oven (32–45°C).

2.2. Soil-exposure apparatus

Five polycarbamate, disposable-type animal cages were used as soil-holding boxes ($46.9 \times 26.7 \times 20.3$ cm; Allentown Caging, Allentown, NJ). The tops (soil surfaces) were open to the air. A plastic grid, $40 \times 20 \times 1$ cm, was supported above the bottom using four, 2.5×3.5 cm rubber stoppers. The grid was then covered with wire window screen material (50×30 cm) to reduce soil loss. A 7-cm o.d. hole was drilled through the bottom of each box to collect percolated water in a drip pan.

2.3. Capsicum oleoresin

The capsicum oleoresin was obtained as a dark red liquid (Penta International Corp., Livingston, NJ; Lot Nos. 46051 and 52577); this material assayed at 1,000,039 Scoville units and contained 4.92% capsinoids (Hoffman et al., 1983). Available physical properties include: insoluble in water, soluble in ethanol, ~967.7 g/l density, and 62–65°C melting point.

2.4. Procedures

Each of the boxes was filled with 8500 g of dried soil. Soil was reconstituted based on 15% (1275 ml) gravimetric soil moisture. Briefly, capsicum oleoresin was substituted for appropriate amounts of water to make respective 40%, 20%, 10%, 5%, and 0% liquid concentrations: 40% = 765 : 510 ml, 20% = 1020 : 255 ml, 10% = 1147 : 127.50 ml, 5% = 1211.25 : 63.75 ml, and 0% = 1275 : 0000 ml water : capsicum oleoresin, respectively. These were then added to the soil in respective boxes and mixed thoroughly for ~5 min using a small garden trowel—until the soil had a uniform moist appearance. Resultant soil mixtures contained 6.00%, 3.00%, 1.50%, 0.75%, and 0.00% capsicum oleoresin (wt : vol).

Next, the 5 boxes of soil were transported outdoors and placed on a wooden pallet. The boxes were covered with clear plexiglass sheets ($75 \times 40 \times 0.6$ cm) that were elevated ~10 cm above the boxes using pieces of construction lumber; this allowed air flow over the soil surface and prevented natural precipitation from reaching the soil. Boxes were positioned ~20 m west of our laboratory; this location afforded excellent sunlight, but afforded protection of the boxes from excessive wind. Minimum vs. maximum temperature readings recorded at an official weather station located ~1 km from the site during the 28-day period of the study were 12°C vs. 34°C (13°C vs. 34°C for Days 1–14 and 12°C vs. 31.5°C for Days 15–28); external precipitation (outside of soil boxes) totaled 7.14 cm and occurred on 14 days of the study (i.e., 6 days of the Day 1–14 period = 1.45 cm; 8 days of the Day 15–28 period = 5.69 cm. Although actual temperatures within the soil boxes were not recorded, minimum and maximum temperatures recorded on the surface of the plexiglass rain protector during the 28-day period were 11.5°C and 48.5°C, respectively.

To simulate rainfall, ~800 ml (0.64 ml/cm^2) of water was sprinkled onto each box daily for 14 days using a sprinkling can. Percolating liquid from each box was caught in a metal drip pan.

Immediately after mixing and prior to watering on Days 2, 4, 6, 8, 10, 12, and 14, plus on Day 28 (no watering), a 25 g sample of soil was taken from each half of each soil box (depths of ~15 cm) and placed onto a 12.7-cm^2 plastic weigh boat. These were dried at 42–48°C for 24 h in a laboratory oven. Ten gram portions were weighed from each of the samples and placed into separate, pre-labeled 50-ml Falcon tubes. Fifty milliliters of reagent-grade methanol were added to each tube, and this mixture was shaken by hand vigorously for 3 min and centrifuged at 3000 rpm for 3 min. The methanol/capsaicin extract was poured off into pre-labeled 250 ml Erlenmeyer flasks. Next, another 50 ml of methanol was added to the soil samples, which were again shaken and centrifuged for 3 min. The extract was added to the previous one in respective flasks. A 1-ml aliquot of the extract was withdrawn and then added to 14 mls of methanol. This process resulted in a 1 : 15 dilution ratio of the extract.

Dual 1-ml aliquots of extract from the independent soil samples were subsequently analyzed for capsaicin content using a Hewlett-Packard Model 8451-A spectrophotometer. An empty quartz cuvette was used as a reference for a cuvette of pure methanol. Methanol absorbance was scanned at wavelengths between 190 and 820 nm. This spectrum was obtained mainly as a check for potential contaminants, and the methanol sample was, in turn, used as a reference for the soil extracts. This procedure preceded every batch of soil–sample extracts that were analyzed. Soil extracts were analyzed by using 1-ml aliquots in a clean cuvette. The absorption maximum (λ_{max}) was recorded for each extracted sample at 282 nm (Owen, 1988).

2.5. Data analyses

Separate linear regression analyses (Program REG, SAS Institute, 1990) were computed for the mean λ_{max} readings of the Day 0 (initial mix), 2, 4, 6, 8, 10, 12, and 14 extracts (i.e., simulated rainfall days) from each capsicum–soil mixture. Spectrophotometric readings for the final, Day 28 extracts were characterized descriptively; these values reflected the status of capsicum oleoresin residues after another 14 days in the absence of simulated rainfall. Additionally, PROC GLM (SAS Institute, 1990) was used to test the separate-slopes model (slope differences) among the absorbance data from Days 0, 2, 4, 6, 8, 10, 12, and 14 oleoresin extracts for the four oleoresin regression functions.

3. Results

Mean spectrophotometric readings of extracts from the 5 capsicum oleoresin soil mixtures increased transitively as a function of concentration (Table 1). Readings declined for the respective capsicum–soil concentrations as 0.64 cm/day water was sprinkled on the soil during Days 1–14, then remained stable (or increased slightly) in the absence of further simulated rainfall during Days 15–28 (Fig. 1). Mean (±SD) absorbance values for 6.00%, 3.00%, 1.50%, 0.75%, and 0.00% soil mixtures at Days 14 and 28 were 0.666 (±0.075), 0.198 (±0.055), 0.131 (±0.007), 0.030 (±0.001), and −0.021 (±0.001) and 0.750 (±0.031), 0.271 (±0.028), 0.105 (±0.027), 0.045 (±0.000), and −0.001 (±0.002), respectively. Thus, extracts from the capsicum soils showed decreases of ⩾45% A.I. after 14 successive days of simulated rainfall, while 3 of the 4 capsicum–soil mixtures displayed modest increases in absorbance after 14 more days without watering.

Computed half-life equivalents based on the absorbance readings were: 0.5991, 0.3572, 0.1312, 0.0751 for the 6.00%, 3.00%, 1.50%, and 0.75% capsicum–soil mixtures, respectively. Although the 6.00% mixture never reached the half-life value, estimates for the 3.00%, 1.75%, 0.75% mixtures were met by Day 2, 14, and 8 of the soil-exposure period, respectively.

Except for the 0.0% (control) wt : vol capsicum mixture, all linear regression equations for soil extracts during the first 14-days (simulated rainfall) yielded F ratios indicative of linear fits that differed from no regression (Table 1). Cumulative simulated rainfall was inversely predictive of optical density at 282 nm for most capsicum soil mixtures. The functions for 6.00%, 1.50%, and 0.75% capsicum accounted for a maximum of 65–75% of the variance in the data sets; whereas, the extractions from the 3.0% capsicum mixture accounted for only 43% of this mixture's spectrophotometric variation. The lack of regression for our control (0.00%) mixture was expected; this attests to the lack of bias in our methods.

Table 1
Day 1 (post mixing, pre-watering samples) capsicum oleoresin analysis data

Soil mixture (%)	Mean (±SD) UV absorbance (AU)	Coefficient of variation (%)	Response factor[a]
6.00	1.200 (±0.064)	5.3	5.0
3.00	0.714 (±0.017)	2.4	4.2
1.50	0.262 (±0.045)	17.0	5.7
0.75	0.150 (±0.032)	21.0	5.0
0.00	−0.009 (±0.004)	n/a	n/a

[a]Soil–capsicum concentration UV absorbance.

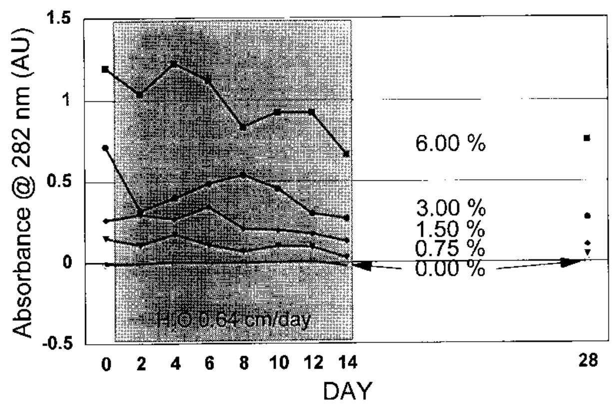

Fig. 1. Time course of mean absorbance readings (λ_{max} at 282 nm) for dual extracts of respective 6.00%, 3.00%, 1.50%, 0.75%, and 0.00% capsicum–soil mixtures (simulated rainfall was applied at the rate of 0.64 cm/day for the first 14 days post mixing, then soils were kept dry for an additional 14 days).

Scatter plots of these spectrophotometric readings and simulated rainfall data confirmed that cumulative water was an important regressor of capsicum oleoresin depletion from the sandy loam soil (Fig. 2). Intercepts of these functions increased proportionately with wt : vol concentrations, but the negative slopes were gradual, irrespective of capsicum oleoresin concentration. The comparison of slopes among the 4 oleoresin regressions yielded significance ($F_{7,24} = 66.59$, $p = 0.0001$; $R^2 = 95$)—heterogeneous slopes. This was evident in the slightly greater absorbance slopes that occurred with increasing oleoresin concentrations (Table 2).

4. Discussion

We chose to monitor persistence of capsicum oleoresin in the soil samples by measuring UV absorbance of soil extracts at λ_{max} of capsaicin. To permit this, we held extraction and dilution volumes constant for all extracts as well as spectrophotometer cell path length. Given also that molar absorptivity of capsaicin is a constant, UV absorbance of the soil extract was directly proportional to soil oleoresin concentration according to Beer's law (Skoog and West, 1982). We employed absorbance measurements in all calculations of soil persistence.

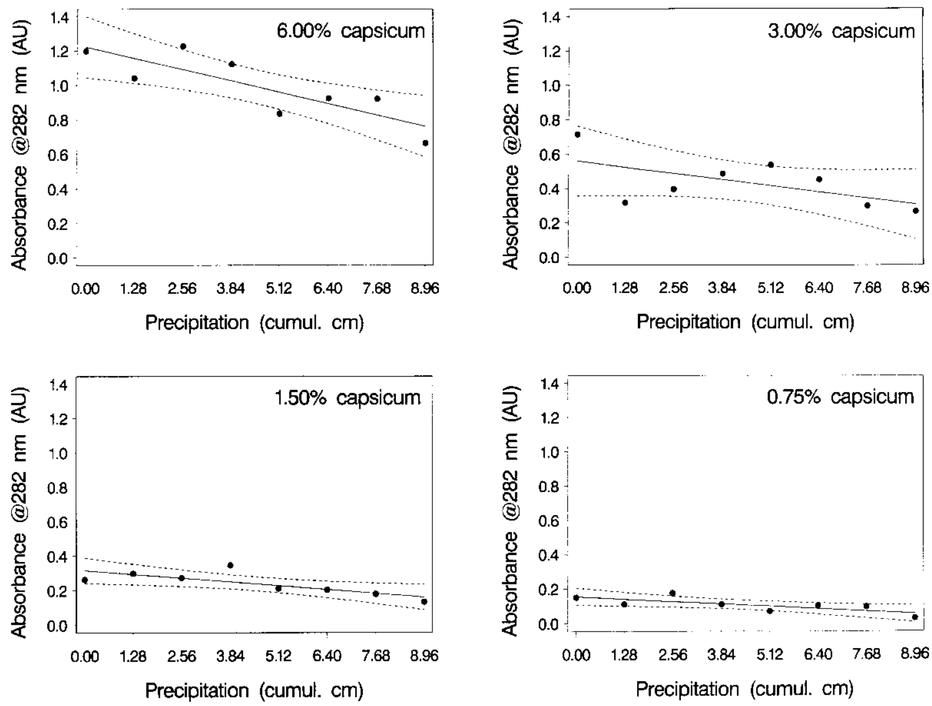

Fig. 2. Four scatter plots of mean absorbance readings (λ_{max} at 282 nm) for dual extracts of 6.00%, 3.00%, 1.50%, and 0.75% capsicum–soil mixtures vs. simulated rainfall (cm) for the initial 14 days post mixing.

Table 2
Regression equations, proportion of predicted variances (R^2), and F ratios for the 6.00%, 3.00%, 1.50%, 0.75%, and 0.00% capsicum oleoresin, soil extracts

Soil mixture (%)	Regression[a]	R^2	$F_{1,6}$ (p)
6.00	$Y = +1.22–0.05X$	0.75	21.28 (0.002)
3.00	$Y = +0.56–0.03X$	0.43	5.25 (0.056)
1.50	$Y = +0.32–0.02X$	0.69	9.55 (0.005)
0.75	$Y = +0.15–0.01X$	0.65	8.32 (0.008)
0.00	$Y = -0.00–0.00X$	0.00	0.05 (0.936)

[a] Y is λ_{max} at 282 nm and X is accumulated precipitation after insertion of capsicum oleoresin in soil (cm).

Evaluation of the data demonstrated the validity of an underlying assumption in our analytical approach, i.e., oleoresin extraction efficiency (recovery) did not vary significantly among replicate extractions or by concentrations. Examination of Day 1 data showed that the coefficients of variation (CV) for dual extractions were excellent at the 6.0% and 3.0% levels (Table 1). Increased variability (see CVs) at lower soil concentrations was likely a factor of sample heterogeneity rather than extraction variability.

Response factors (RFs) were also calculated from Day 1 data by dividing the oleoresin concentration by the mean UV absorbance. Upward or downward trends in RFs with respect to concentration would be indicative of sample concentration influences on oleoresin recovery. The RF data indicated that oleoresin recovery was not a function of oleoresin concentration (see Table 1). Furthermore, spectrophotometric data obtained from the 0.0% samples demonstrated that the matrix did not contribute to UV absorbance.

A complicated depletion function characterized both the capsicum–soil-concentration data and the half-life estimates. Persistence showed mixed results, with the 6.00% and 1.5% capsicum–soil mixtures requiring > 14 days (with or without simulated rainfall) to deplete by half, but the 3.00% and 0.75% soils depleting by 50% in < 8 days or 12 days depending on whether or not the initial half-life value is considered anomalous (variance of absorbance readings). A parsimonious explanation is that the initial decrease with some recovery to higher absorbance was due to soil-mixing and -sampling factors. Upon mixing soils with the capsicum, "clumps" of orange-colored soil formed; this made uniform, homogeneous sampling of soils difficult. Still, the fact that both the 3.0% and 6.0% mixtures showed pronounced decreased absorbance on Day 2 with returns to higher readings by Day 4 makes us wary—more leaching and persistence data involving manipulations of simulated rainfall are needed.

Although we suspect that capsicum oleoresin leaches rapidly in loose, sandy loam soils that receive moderate rainfall, but not in semi-arid and arid soils (and we suspect more compacted soils), we did not analyze the percolates for capsicum. Nevertheless, the initial percolate collections from the mixtures were colored bright orange, with decreasing coloration evident for successive watering trials. Leaching rate is dependent upon adsorption, dispersion, and water infiltration/evaporation (Hamaker, 1975). The fact that we used loose (not compacted) and deep soil, coupled with the rapid application (0.64 cm/day in < 10 min) of simulated rainfall, probably enhanced adsorption, porous flow, and diffusion of the chemical.

Photolysis also was uncontrolled in the study, and this factor remains a potential source of capsicum depletion. Ambient weather records for the period of the soil exposures showed that solar radiation was less during the Day 15–28 no-simulated rainfall period, while the oleoresin was more stable. Mean ($\pm$SD) and minimum–maximum solar radiation data (Langley units; 1 cal/cm^2) for Days 1–14 and 15–28 were 472.6 ($\pm$139.6) and 179–669 vs. 353.6 ($\pm$137.3) and 121–496, respectively (Colorado Ag Meteorological Network, 1999). Additionally, hydrolysis should be minimal due to the oil-based formulation mentioned earlier; reformulating with other non-oil-based carriers would be expected to increase the role of hydrolysis and should affect persistence.

Capsaicin and capsicum oleoresin are generally considered safe chemicals. The chemical is used medicinally in humans as a 0.075% cream prescribed for pain following shingles (US Pharmacopeal Convention, 1994). Still, debilitating respiratory distress, lacrimation, and mucosa burning from concentrates must be noted; and, the inadvertent (but unlikely) consumption of the concentrated A.I. could prove fatal (i.e., probable oral lethal dose = 0.5–5 g/kg; Gosselin et al., 1976).

Concern for dietary and dermal toxicity of capsaicin in both target and non-target terrestrial species (i.e., birds and mammals) is viewed to pose minimal risks because of the irritant's immediate repellent action; but, in the absence of specific aquatic data, the EPA views fish and aquatic species as unable to avoid chemicals that may become mixed or dispensed into their habitats, and a "precautionary statement" warning against runoff of capsaicin-based products into watersheds is required on product-use labels by registrants (Environmental Protection Agency, 1992a).

Regarding the implications of these data to development of the "soil-irritant/repellent" concept for pocket gopher management, research and development efforts continue (Sterner et al., in preparation). Sufficient persistence of capsicum oleoresin, or any irritant for that matter, is crucial to make the technique economical and effective. Capsicum oleoresin sells for ~\$50/kg; this precludes use of the compound as a potential rodent repellent for all except limited applications (e.g., homeowner lawns) or very high-profit ventures (e.g., ornamental nurseries, golf greens). Still, results strongly suggest that single applications of capsicum oleoresin as a model irritant for these studies would persist for several months, possibly a year, in arid and semi-arid locales such as the southwestern US.

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